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PATENT SPECIFICATION

953,899

NO DRAWINGS.

Date of Application and filing Complete Specification :
May 18, 1961. No. 18086/61.

Application made in Germany (No. N19273 IVb/12o) on
Dec. 5, 1960.

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Index at Acceptance :—G2 C(P2E11B, P2E12A, P2E12B, P2E15A, P2E19B, P2E19D, P2E26B, P2E33, P5A, P5B, P7); G2 U(4A1, 4C3, 4, X7).

International Classification :—C 07 f (C 07 c).

COMPLETE SPECIFICATION.

Process for the Preparation of Natural Choline Phosphoric Acid
Diglyceride Ester Compounds.

PATENTS ACT, 1949

SPECIFICATION NO. 953,899

In accordance with the Decision of the Superintending Examiner, acting for the Comptroller-General, dated the 23rd day of October, 1964, this Specification has been amended under Section 29 in the following manner:—

Page 2, line 67, page 4, lines 24 and 42, *delete* "moderately" *insert* "markedly"

Attention is also directed to the following printer's errors:—

Page 1, Index at Acceptance, *for* "4,X7" *read* "4X,7"

Page 1, line 3, *for* "nationals" *read* "Nationals"

Page 1, line 22, *before* "soya" *insert* "from"

Page 2, line 6, *for* "Sorg" *read* "Sorge"

Page 2, line 112, *for* "example" *read* "Example"

Page 4, line 58, *for* "desoxylate" *read* "desoxycholate"

THE PATENT OFFICE,
20th January, 1965

D 33650/6

- 30 basic component a choline ester of diglyceride phosphoric acid and recovering the said phospholipid from the alcohol solution, all steps being carried out in the presence of an inert gas at a temperature not exceeding 35° C. whilst excluding the presence of oxygen and light.

- 35 In order to be able to administer the above mentioned esters more easily, to inject them parenterally and especially intravenously, it was considered desirable to convert these water-insoluble esters into water-soluble

with these complexes an exchange of the vitally necessary lipids or lipoids takes place in the cell metabolism processes. They are irreplaceable in the physiological processes.

Morre and Rockwood (Proc. Roy. Soc., London 1896, 60, 438—442) mention in their reports the solubility of the fats in solutions of bile acids. They were able to demonstrate that oleic acid is dissolved to 4—5% in the bile itself, while stearic acid is much less soluble. When the two acids are mixed, however, the solubility of the mixture is in-

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International Classification :—C 07 f (C 07 c).

COMPLETE SPECIFICATION.

Process for the Preparation of Natural Choline Phosphoric Acid Diglyceride Ester Compounds.

We, ROLF LAPPE, WALTER LAPPE, HANS EIKERMANN and RIA GRIESHABER-LAPPE, all German nationals, trading as the firm A. NATTERMANN & CIE, of Koeln-Braunsfeld, Germany, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

10 This invention relates to a process for the preparation of water soluble choline phosphoric acid diglyceride ester compounds.

15 Natural choline phosphoric acid diglyceride esters with a high content of unsaturated and essential fatty acids and free from colamine phosphoric acid diglyceride esters, are known to possess considerable importance in biology and medicine. These esters may be obtained by the process described and claimed in our copending Application No. 21837/58 (Serial No. 877,031) soya bean crude lecithin comprising extracting said crude lecithin with acetone, extracting the residual ester mixture with alcohol, allowing the alcoholic solution to stand for several days to clarify the solution from suspended colloidal particles and removing the alcoholic solution containing as predominantly the basic component a choline ester of diglyceride phosphoric acid and recovering the said phospholipid from the alcohol solution, all steps being carried out in the presence of an inert gas at a temperature not exceeding 35° C. whilst excluding the presence of oxygen and light.

40 In order to be able to administer the above mentioned esters more easily, to inject them parenterally and especially intravenously, it was considered desirable to convert these water-insoluble esters into water-soluble

compounds, particularly into water-soluble compounds which are stable to a practically unlimited extent, despite their content of unsaturated and essential fatty acids which are particularly subject to oxidative treatment due to their double bonds.

It is known that water-soluble molecular compounds are formed from bile acids and fats, as also lipoids, which are known to include the choline phospholipids. In this connection it will be seen from the following references to the literature that the molecular compounds formed from fats and like compounds on the one hand and bile acids or the like on the other hand have considerable importance for metabolism in the human organism:

In the Lehrbuch der Physiologischen Chemie, Leipzig 1850, 2nd Edition, it was pointed out that the bile acids are capable of fat emulsification. It was furthermore already known at this time that the phospholipids, which occur in the bile, further activate the emulsifying action of the bile acids. The best known bile acids occurring in the liver are able to form molecular compounds with lipids and lipoids. Through these molecular compounds the formerly insoluble substances are made water-soluble. With these complexes an exchange of the vitally necessary lipids or lipoids takes place in the cell metabolism processes. They are irreplaceable in the physiological processes.

Morre and Rockwood (Proc. Roy. Soc., London 1896, 60, 438—442) mention in their reports the solubility of the fats in solutions of bile acids. They were able to demonstrate that oleic acid is dissolved to 4—5% in the bile itself, while stearic acid is much less soluble. When the two acids are mixed, however, the solubility of the mixture is in-

creased to 15%. By adding a large portion of Na_2CO_3 the solubility could even be doubled. If the free fatty acids were replaced by neutral fats, the solubility became appreciably less.

H. Wieland and H. Sorg (Z. physiol. Chem., 1916, 87, 1—27) first introduced the concept of the taurocholic acids. These relate to addition compounds of fatty acids to acids of the bile acid group. The two authors found that a true complex formation only takes place with desoxycholic acid and apocholic acid. Desoxycholic acid is a saturated acid with two hydroxyl groups, a dihydroxycholanolic acid; apocholic acid is an unsaturated acid also with two hydroxyl groups, a dihydroxycholenic acid. The other bile acids also form addition compounds. Biologically the adducts of desoxycholic acid are of most importance.

Thus J. C. M. Verschure (Clin. chim. acta, Amsterdam, 1956, 1, 511—518), by paper electrophoresis and investigations in the ultracentrifuge, could detect a high molecular complex from lecithin and desoxycholate occurring in the human gall bladder. The components were present in the ratio 1:8.

A. E. Meyer and J. P. McEwen (Am. J. Physiol., 1948, 153, 386—392) ascertained that even desoxycholic acid and cholic acid, when they are brought on the surface of cell walls, are able to bring about metabolic processes by their chemical molecular compounds. They even affect the peristaltic movements. Desoxycholic acid was known as the most active acid. It was able to form the macromolecular taurocholic acid.

H. Rheinboldt and co-workers (Ann., 1927, 451, 256—273 and Ann., 1929, 473, 249—259) also investigated the taurocholic acids and the systems of substances associated with them. They obtained, from an alcoholic solution of desoxycholic acid and stearic acid, a molecular compound which has a sharp melting point. The compound was so stable that it could not be split either by hydrogenation or by oxidation.

Rheinboldt was able to show that the bile acids form molecular compounds not only with fatty acids, but also with alicyclic acids or their compounds which have a long side chain.

From the known state of the art it is clear that relatively large amounts of bile acid salts are necessary in order to form water-soluble compounds from the latter and, for example, lecithin, 8 mol of bile acids to each mol of lecithin being in fact required.

According to the present invention there is provided a process for the preparation of water-soluble choline phosphoric acid diglyceride ester compounds which comprises reacting natural choline phosphoric acid diglyceride esters free of colamine phos-

phoric acid diglyceride esters and having a moderately preponderant proportion of essential and other unsaturated fatty acids in relation to the total fatty acid content with an aqueous solution of sodium desoxycholate in an alkaline medium and in the presence of an inert gas, less than 1 mol sodium desoxycholate being used for 1 mol of diglyceride ester. The lowest ratio is about 0.8 mol of said sodium desoxycholate per mol of diglyceride ester. A preferred inert gas is nitrogen.

The diglyceride esters which can be prepared in accordance with U.K. Patent 877,031, comprise in relation to the total fatty acid content, e.g., more than 80.0% of unsaturated fatty acids of which the greatest part consists of essential fatty acids. To give an example: in a content of 82.3% of unsaturated fatty acids the oleic acid content is 17.2%, the linoleic content is 52%, and the linolenic acid content is 13.1%.

The pH value of the reaction mixture is preferably above 8; it can, if necessary, be corrected with N-NaOH. As is known the natural choline phosphoric acid diglyceride esters are themselves very alkaline.

A clear aqueous solution results and only long-chain fatty acids are present in the esters.

Moreover up to 50% by weight of the diglyceride ester fraction may be replaced by lysolecithin, in which case the quantity of the bile acids salt required for the process of the present invention is still further reduced. For this purpose a lysolecithin is advantageously used which is obtained by the process disclosed in our copending Application 21837/58 (Serial No. 877,031) from the choline phosphoric acid diglyceride esters. Such a lysolecithin has about 28% of essential fatty acids in relation to the total fatty acids.

According to a further embodiment of the invention the very dilute alcoholic solution such as is described in the example of our copending Application 21837/58 (Serial No. 877,031) which contains about 1% of diglyceride esters may be employed as the initial material when it is treated as such with a small quantity of alpha-tocopherol to form an adduct therewith before the alcohol is distilled off under reduced pressure in the presence of an inert gas, such as nitrogen or CO_2 , after which the reaction of the adduct in the form of a pasty mass from the diglyceride esters and alpha-tocopherol with sodium desoxycholate as indicated above is carried out. It has in fact been surprisingly found that the reaction of the adduct with the salt of bile acids yields a water-soluble compound, although as is known the alpha-tocopherol itself is not soluble in water. For the purposes of the

invention alpha-tocopherol acetate is preferred because of its comparative stability.

The alpha-tocopherol serves not only for the protection of the unsaturated, especially the essential, fatty acids to oxidation, but the substance known as vitamin E possesses also a biological-therapeutic intrinsic value which is of additional medicinal significance in the product of the process of the present invention. For the process of the invention quantities of alpha-tocopherol are sufficient which are of the order of magnitude of 0.2% per gram of choline phosphoric acid diglyceride esters; larger amounts, however, may also be used when this is desired on medical grounds. Up to 2% by weight of alpha-tocopherol may be added to the diglyceride esters or to the mixture of diglyceride esters and lysolecithin; the addition product forms a water-soluble complex with the salt of bile acids.

The invention will now be further described by way of examples.

EXAMPLE 1.

600 g. of natural choline phosphoric acid diglyceride esters comprising in relation to the total fatty acids present as ester constituents more than 80% of essential and other unsaturated fatty acids and free from colamine phosphoric acid diglyceride esters are treated with 500 ml. of a sodium desoxycholate solution prepared as given below, in an atmosphere of nitrogen with continuous stirring. A viscous mass is formed. The remaining quantity of sodium desoxycholate solution is added over a period of 1½ hours. After all the reaction and solution processes are completed, it is made up to the final concentration (12 litres) with water adjusted to a pH value of about 7.8 with N-NaOH.

EXAMPLE 2.

60 litres of a 1% alcoholic solution of natural choline phosphoric acid diglyceride esters such as are used according to Example 1, are treated with 1.32 g. of alpha-tocopherol acetate. The alcoholic solution may be prepared as given below. After the alpha-tocopherol acetate has gone completely into solution, the alcohol is distilled off under reduced pressure in the presence of an inert gas, and a product of a pasty nature is obtained. 600 g. of this are treated with 500 ml. of a sodium desoxycholate solution prepared as given below, in an atmosphere of nitrogen with continuous stirring. A viscous mass is formed. The remaining quantity of desoxycholic acid solution is added over a period of 1½ hours. After all the reaction and solution processes are completed, it is made up to the final concentration (12 litres) with water adjusted to a pH value of about 7.8 with N-NaOH.

Preparation of sodium desoxycholate solution.

240 g. of desoxycholic acid are suspended in 2 litres of water and slowly treated with a NaOH solution (24 g. of NaOH in 500 ml. of water) while boiling until a clear solution is obtained. After the solution is quite cold the pH value is checked and if necessary corrected to about 8 with N-NaOH.

Preparation of alcoholic solution of diglyceride esters.

The alcoholic solution of natural choline phosphoric acid diglyceride esters with a high content of unsaturated and essential fatty acids and free from colamine phosphoric acid diglyceride esters used for the process of the invention may be prepared, for example, as follows:—

8 kg. of crude soya bean phosphatides are repeatedly extracted with 150 litres of acetone at a temperature not exceeding 35° C., with exclusion of light and oxygen in order to free them from oily constituents. The residue is freed from acetone in vacuo and in the presence of an inert gas, after which the residue is extracted several times with ethanol, again at a temperature not exceeding 35° C. and with exclusion of light, and air or oxygen, while an inert gas is passed through the extraction apparatus. The alcoholic solutions obtained in the extraction are combined and allowed to stand for several days in order to free them from suspended or colloiddally dissolved substances therein, which settle and are removed by filtering or decanting the alcoholic solution. One litre of the alcoholic solution obtained in this way, which contains substantially choline phosphoric acid diglyceride esters, is diluted to 2—3% with alcohol and, over a period of 10 hours with exclusion of light and air or oxygen is passed through an absorption column of, for example 4 cm. in diameter, in which is a suspension of 10% of magnesium oxide and aluminium oxide in ethanol. After the alcoholic solution has passed through the column, the latter is eluted with 3 litres of ethanol. The alcoholic solutions obtained contain natural choline phosphoric acid diglyceride esters free from colamine phosphoric acid diglyceride esters, and containing at least 66% of essential fatty acids referred to the total amount of fatty acids present.

Where lysolecithin is to be used, it may be prepared from the alcoholic solution of the natural choline phosphoric acid diglyceride esters free from colamine phosphoric acid diglyceride esters by the concentration in vacuo in the presence of an inert gas. 5.7 g. of these choline esters are dissolved in 570 c.c. of peroxide-free ether, treated with 6 c.c. of an aqueous solution of 18 mg. of snake venom (*Crotalus adamanteus*) and

after repeated shaking up, are allowed to stand for 24 hours at room temperature. Then disregarding the sediment formed, the solvent is distilled off in vacuo. 2.9 g. of choline esters of the monoglyceride phosphoric acid (lysolecithin) are obtained, which contains 28% of polyunsaturated essential fatty acids, calculated on the total fatty acids.

The water soluble choline phosphoric acid diglyceride ester compounds prepared in accordance with the present invention are very helpful means for curing arteriosclerosis and diabetes. The compounds are preferably used in the form of a 5 or 10% aqueous solution which are applied parenterally e.g. intravenously.

WHAT WE CLAIM IS:—

1. Process for the preparation of water-soluble choline phosphoric acid diglyceride ester compounds which comprises reacting natural choline phosphoric acid diglyceride esters, free of colamine phosphoric acid diglyceride ester and having a moderately preponderant proportion of essential and other unsaturated fatty acids in relation to the total fatty acid content, with an aqueous solution of sodium desoxycholate in an alkaline medium and in the presence of an inert gas, less than 1 mol but not less than 0.8 mol, of sodium desoxycholate being used for 1 mol of the diglyceride ester.

2. Process as claimed in Claim 1 in which the inert gas is nitrogen.

3. Process as claimed in Claim 1 or Claim 2, in which lysolecithin replaces up to 50% by weight of the choline phosphoric acid diglyceride esters.

4. Process as claimed in Claim 3, in

which the lysolecithin is obtained from the natural choline phosphoric acid diglyceride esters having a moderately preponderant proportion of essential and other unsaturated fatty acids in relation to the total fatty acid content and free from colamine phosphoric acid diglyceride esters by the process disclosed in Specification No. 877,031.

5. Modification of the process claimed in any preceding claim in which a very dilute alcoholic solution of said diglyceride esters as described in the Example of Specification 877,031 is treated with alpha-tocopherol to form an adduct therewith the alcohol being subsequently removed under reduced pressure in the presence of an inert gas, and said adduct being then reacted in alkaline medium with an aqueous solution of sodium desoxylate wherein less than 1 mol but not less than 0.8 mol, of sodium desoxycholate is used per mol of the diglyceride ester.

6. Process as claimed in Claim 5 in which the quantity of alpha tocopherol is 0.2% by weight per gram of choline phosphoric acid diglyceride ester.

7. Process for the preparation of water-soluble choline phosphoric acid diglyceride ester compounds substantially as described herein, with particular reference to the examples.

8. Water soluble choline phosphoric acid diglyceride ester compounds when prepared in accordance with the process claimed in any of the preceding claims.

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